UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 09/757,054 | 01/08/2001 | James N. Petitte | 297/93/2 | 7757 |

25297 7590 04/11/2007

JENKINS, WILSON, TAYLOR & HUNT, P. A.
3100 TOWER BLVD
SUITE 1200
DURHAM, NC 27707

| EXAMINER | |
|---|---|
| WILSON, MICHAEL C | |
| **ART UNIT** | **PAPER NUMBER** |
| 1632 | |

| SHORTENED STATUTORY PERIOD OF RESPONSE | MAIL DATE | DELIVERY MODE |
|---|---|---|
| 2 MONTHS | 04/11/2007 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov



**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Application Number: 09/757,054
Filing Date: January 08, 2001
Appellant(s): PETITTE ET AL.

Arles Taylor
For Appellant

**EXAMINER'S ANSWER**

This is in response to the appeal brief filed 10-27-06 and corrected 11-28-06 appealing from the Office action mailed 12-30-05.

**(1) Real Party in Interest**

A statement identifying by name the real party in interest is contained in the brief.

**(2) Related Appeals and Interferences**

The examiner is not aware of any related appeals, interferences, or judicial proceedings that will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

**(3) Status of Claims**

The statement of the status of claims contained in the brief is correct.

**(4) Status of Amendments After Final**

No amendment after final has been filed.

**(5) Summary of Claimed Subject Matter**

The summary of claimed subject matter contained in the brief is correct.

**(6) Grounds of Rejection to be Reviewed on Appeal**

The appellant's statement of the grounds of rejection to be reviewed on appeal is substantially correct. The changes are as follows:

A. Claims 53 and 54 stand subject to a new matter rejection because the specification as filed did not contemplate maintaining the ES cell phenotype for one or two months as claimed.

B. Claims 44, 47, 48, 51-54 and 56-58 stand subject to an enablement rejection because the specification as filed did not provide adequate guidance for those of skill to determine the specific combination of parameters required to sustain ES

cells in culture or maintain the ability to contribute to germ and somatic cells upon being introduced into recipient embryo for one or two months as claimed.

C. Claims 44, 47, 48, 51-54 and 56-58 stand rejected as being indefinite because

i) the phrase "undifferentiated chicken cells expressing an embryonic stem cell phenotype" in claim 44 is unclear.

ii) it is unclear how PGCs isolated from an embryo later than stage 14 are distinguished from PGCs isolated from a stage X or stage 14 embryo as in claims 44, 47 and 48.

Items D-F in the Appeal Brief are correct.

G. Claims 44, 47, 48, 52-54 and 58 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Ponce de Leon (US Patent 6,156,569) in view of Chang (1995, Cell Biol. Internat'l, Vol. 19, page 143-149).

H.

Item I in the Appeal Brief is correct.

**(7) Claims Appendix**

The copy of the appealed claims contained in the Appendix to the brief is correct.

**(8) Evidence Relied Upon**

Simkiss (1990, 4th World Congr. Genetic Appl. Livestock Prod., Vol. 16, pg 111-114)

Petitte (1990, Development, Vol. 108, pg 185-195)

Ponce De Leon (1997, Revista Brasileira de Reproducao Animal, Vol. 21, pg 96-101)

Naito (Mol. Reproduction and Develop., 1994, Vol. 37, pg 167-171)

Chang (1995, Cell Biol. Internatl. Vol. 19. No. 2, pg 143-149)

Chang (1997, Cell Biol. Internatl., Vol. 21, No. 8, pg 495-499)

Petitte (US Patent 5,340,740)

Petitte (US Patent 5,656,479)

Petitte (US Patent 5,830,510)

Ponce de Leon (US Patent 6,156,569)

Pain (1996, Development, Vol. 122, pg 2339-2348)

It is noted that Pain (1996) has been considered for art purposes and is considered the closest prior art as far as the structure and function of the culture claimed (the process limitation of isolating PGCs from an embryo after stage 14 does not distinguish the PGCs claimed from the PGCs inherently found in the dissociated whole stage X embryo described by Pain). However, Pain (1996) did not teach dissociated whole stage X embryos comprised fibroblasts or any other stromal cells, nor is it readily apparent that dissociated whole stage X embryos comprised fibroblasts or any other stromal cells.

**(9) Grounds of Rejection**

The following ground(s) of rejection are applicable to the appealed claims:

A. Claims 53 and 54 stand rejected under 35 U.S.C. 112, first paragraph, as containing new matter because the specification did not contemplate maintaining an ES cell phenotype for one or two months.

Pg 13, line 21, through pg 14, line 7 (cited on pg 14-15 of the response filed 1-2-04) teaches:

> "In a preferred embodiment, avian embryonic gonadal cells comprising primordial germ cells from a four to five day incubated avian embryo are seeded onto the preconditioned feeder matrix with conditioned media, and the avian cells give rise to nests or colonies of cells exhibiting an embryonic stem cell pheno [sic]Unlike the case with mammalian cells, it is currently preferred to have a preconditioned feeder matrix to facilitate the survival and development of avian PGCS into undifferentiated avian cells expressing an ESC phenotype. The avian embryo cells of the present invention can be cultured for at least one or two months as is typical for a primary cell culture, which is significantly greater than the usual two week life of primary cultures of cells from an unincubated avian embryo." (underlining added)

The citation contemplates culturing undifferentiated avian cells having an ES cell phenotype and culturing avian embryo cells for at least one or two months. The citation does not imply the ES cell phenotype is maintained for one or two months as now claimed. While "cells expressing an ESC phenotype" may be obtained and "cells of the invention can be cultured for at least one or two months," it is not readily apparent from those two sentences alone, taken with the rest of the paragraph, or taken with the rest of the specification, that applicants contemplated maintaining the ESC phenotype for one or two months. Maintaining the ES cell phenotype for at least one or two months as now claimed encompasses maintaining the ES cell function, i.e. the ability to contribute to germ and somatic cells upon being introduced into a recipient embryo, for one or two months, which is broader than the scope described on pg 13-14 and not readily

apparent or implied from the scope described on pg 13-14. Accordingly, maintaining the ES cell phenotype for one or two months as in claims 53 and 54 is new matter because it has a different scope than the teachings on pg 13-14 and because the claims now encompass embodiments broader than those contemplated in the specification as originally filed.

B. Claims 44, 47, 48, 51-54 and 56-58 stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a culture comprising chicken ES cells does not reasonably provide enablement for a culture wherein ES cells are maintained for one or two months.

**Claims/breadth**

Claim 44 is drawn to a sustained culture of undifferentiated chicken cells expressing an embryonic stem cell phenotype, comprising a preconditioned feeder matrix, conditioned media, and chicken primordial germ cells and chicken stromal cells, wherein the chicken primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from an chicken embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system and grown in the sustained culture to produce undifferentiated chicken cells expressing an embryonic stem cell phenotype.

Claims 53 and 54 are drawn to the sustained culture of claim 44, wherein the ES cell phenotype is maintained for at least one or two months.

An undifferentiated avian cell expressing an ES cell phenotype as claimed encompasses undifferentiated chicken cells capable of becoming both a somatic and

germ cell upon being introduced into an embryo. A sustained culture of cells as claimed encompasses a culture of undifferentiated chicken cells having the ability to become both somatic and germ cells upon being introduced into an avian embryo that is sustained for one or two months.

Pain (1996, Development, Vol. 122, pg 2339-2348) described avian ES as being capable of participating in the development of all cell lineages including the germline upon being implanted into a recipient blastocyst (pg 2339, col. 1, line 12; sentence bridging col. 1-2). Applicants' acknowledge that ES cells form somatic and germ cells upon being introduced into a recipient embryo (Petitte, US Patent 5,656,479, Aug. 12, 1997, col. 1, lines 25-27). Thus, the art at the time of filing defined ES cells as being capable of forming somatic and germs upon being injected into a blastocyst. However, the specification states, "embryonic stem cell phenotype refers to undifferentiated avian cells having a large nucleus, prominent nucleolus and little cytoplasm" (pg 9, lines 4-5). Applicants' definition of the ES cell phenotype is repugnant to the art because it does not acknowledge the essential functional definition known in the art – the ability to become both a somatic and germ cell upon being introduced into an embryo. Furthermore, the definition on pg 9 fails to define the metes and bounds of "large nucleus, prominent nucleolus and little cytoplasm." Since the sole disclosed purpose for cells having an ES cell phenotype is to make transgenic avians, taken with the ambiguous metes and bounds of "large nucleus, prominent nucleolus and little cytoplasm" those of skill would rely on the art recognized definition of ES cells.

Therefore, cells having an "ES cell phenotype" as claimed are limited to those capable of becoming both a somatic and germ cell upon being introduced into an embryo.

Reference by the examiner in the enablement rejection to a chicken ES cell is limited to a chicken cell capable of becoming both a somatic and germ cell upon being introduced into an embryo.

**State of the art/unpredictability**

Simkiss (1990, 4th World Congr. Genetic Appl. Livestock Prod., Vol. 16, pg 111-114) and Petitte (1990, Development, Vol. 108, pg 185-195), both of record, taught chicken PGCs capable of producing somatic and germ cell chimeric chickens.

Ponce De Leon of record (1997, Revista Brasileira de Reproducao Animal, Vol. 21, pg 96-101) taught PGCs that provide germ cell chimeras upon being introduced into recipient embryos (pg 100, "Results and Discussion," lines 1-7). Ponce De Leon discusses "long-term culture" of the PGCs isolated from Stage 13 or 14 embryos using LIF, bFGF, IGF and SCF (pg 99, "Materials and Methods," col. 1; pg 100, col. 2, about half way down) and described transferring cells cultured for 25 days into recipient embryos and having progeny tests "available in the next two months" (last few lines of pg 100). "None of the cell feeder layers evaluated in this study improved the long term culture conditions of the PGCs" (pg 100, col. 2, "PGC culture conditions"). Ponce De Leon started the test to determine whether the PGCs cultured for 25 days had the ES cell phenotype but states the results were not available yet. Thus, Ponce De Leon does not teach whether the cells maintained for 25 days had the ES cell phenotype.

Nowhere did the art teach how to maintain the ES cell phenotype of undifferentiated chicken cells in culture for one or two months.

Since the time of filing, Van de Levoir (Methods in Enzymology, 2006, Vol. 418, pg 38-64) taught ES cells derived from blastodermal cells could be cultured for several weeks, but they were incapable of contributing to somatic tissues after more than 3 weeks in culture (pg 39, lines 1-9).

**Teachings in the specification/Examples/amount of experimentation**

The specification taught culturing chicken PGCs on "preconditioned" STO feeder cells (Examples 1-3). The specification suggests the "avian embryo cells of the present invention can be cultured for at least one or two months as is typical for a primary cell culture" (pg 14, lines 4-5). The citation on pg 14, line 4-5, does not describe how to maintain the ES cell phenotype for one to two months as claimed. The specification does not teach the amounts of essential growth factors required to culture chicken ES cells in the presence of feeder cells for one or two months. The specification does not exemplify maintaining the ES cell phenotype for at least one or two months. Given the teachings in the specification and in the art at the time of filing and since the time of filing, it would have required those of skill undue experimentation to determine how to make a culture of undifferentiated chicken cells having the ability to become both somatic and germ cells upon being introduced into an avian embryo that is sustained as broadly claimed in claim 44 or for one or two months ES cells specifically claimed in claims 53 and 54.

**Analysis**

The specification does not enable a culture of undifferentiated chicken cells having the ability to become both somatic and germ cells upon being introduced into an avian embryo that is sustained for more than three weeks as encompassed by claim 44 or for at least one or two months as specifically claimed in claims 53 and 54.

In view of the dearth of information in the art at the time of filing regarding how to maintain undifferentiated chicken cells having the ability to become both somatic and germ cells upon being introduced into an avian embryo for more than three weeks, the parameters required to sustain such cells for more than three weeks are essential to the invention. Because the specification does not teach the essential elements required to sustain ES cells for more than three weeks, the amount of experimentation required by one of skill to obtain such results is, by its very nature, undue. Examples 1, 2 and 3 merely reiterate parameters known in the art such as feeder cells that Ponce De Leon stated failed to improve cell culture. Pg 4, line 18-20, pg 8, lines 20-22 and pg 12, lines 4-8, merely list avian species. The teachings cited in the specification are inadequate to overcome the unpredictability and sustain undifferentiated chicken cells having the ability to become both somatic and germ cells upon being introduced into an avian embryo for more than three weeks. The generic lists of possible parameters described in the specification taken with the mere suggestion of maintaining "embryo cells of the present invention..... ...for at least one or two months" is not a reasonable amount of guidance because the number of combinations contemplated is vast. The specification does not provide the blazemarks required to maintain the ability to become both somatic and germ cells upon being introduced into an avian embryo in undifferentiated chicken

cells for one or two months. Testing the various, numerous combinations of culture conditions to determine those capable of maintaining the ES cell phenotype for one or two months is undue.

C. Claims 44, 47, 48, 51-54 and 56-58 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for reasons of record.

**i)** The cells encompassed by the phrase "undifferentiated chicken cells expressing an embryonic stem cell phenotype" are unclear (claim 44). The specification states, "embryonic stem cell phenotype refers to undifferentiated chicken cells having a large nucleus, prominent nucleolus and little cytoplasm" (pg 9, lines 4-5). The scope of such cells ambiguous because it cannot be determined what applicants consider "large," "prominent" or "little." The description is also ambiguous because a "phenotype" cannot be defined as "cells." In addition, the phrase "refers to," makes the citation even more unclear because it cannot be determined if "refers to" is intended to define the phenotype or merely to describing to what the phenotype is relevant. Therefore, one of skill would not be able to use the description on pg 9 to define the metes and bounds of undifferentiated cells had an "ES cell phenotype."

One of skill would have recognized the definition of an ES cell phenotype on pg 9 was so ambiguous that they would reasonably look to the art to define the phrase. One of skill in the art at the time of filing would have known that ES cells were defined as cells having the ability to become both somatic and germ cells upon being introduced into an avian embryo. One of skill could have reasonably used the art-recognized

definition of ES cells to interpret the phrase "an ES cell phenotype." However, the description on pg 9 has a different scope than the art-recognized definition of ES cells. The specification does not provide adequate guidance to determine the metes and bounds of the definition on pg 9 and does not discuss how those of skill should incorporate the art-recognized definition of ES cells when interpreting the phrase. Accordingly, it is unclear whether "undifferentiated cells expressing an ES phenotype" encompasses a) undifferentiated cells having a large nucleus, a prominent nucleolus and little cytoplasm; b) cells able to contribute to both somatic and germ cells upon being introduced into a recipient embryo (ES cells); or c) both a) and b).

To complicate matters, one of skill would have also recognized the definition of an ES cell phenotype on pg 9 was so ambiguous that they would use the plain meaning of the phrase "an ES cell phenotype." That is to say, one of skill would have reasonably interpreted the phrase as undifferentiated cells having at least one ES cell phenotype, such as a large nucleus, a prominent nucleolus, little cytoplasm, the ability to contribute to germ cells upon being introduced into a recipient embryo or the ability to contribute to somatic cells upon being introduced into a recipient embryo. Using this even broader interpretation, it is unclear whether "undifferentiated cells expressing an ES phenotype" encompasses undifferentiated cells:

a) having a large nucleus; b) having a prominent nucleolus; c) having little cytoplasm; d) able to contribute to somatic cells upon being introduced into a recipient embryo, e) able to contribute to germ cells upon being introduced into a recipient

embryo (PGCs); or f) able to contribute to both somatic and germ cells upon being introduced into a recipient embryo (ES cells).

The specification also states an "'undifferentiated chicken cell expressing an embryonic stem cell phenotype' encompasses cells derived from chicken primordial germ cells and is therefore used to describe the cells cultured in accordance with the process of the present invention" (pg 9, lines 19-22). This sentence does not resolve the issue at hand because the scope of cells encompassed by the description on pg 9, lines 4-5, is different than the scope of the cells encompassed by the description on pg 9, lines 19-22. One of skill would not be able to determine whether to use pg 9, lines 4-5, or pg 9, lines 19-22, as the definition of "chicken cells expressing an ES cell phenotype" as claimed. Furthermore cells do not "express" a phenotype as on pg 9, line 19. Pg 1, line 17, states ES cell were capable of making germline chimeras.

The phrase "embryonic stem cell phenotype" is also mentioned on pg 3, lines 4-5, but does not clarify the meaning.

**ii)** Claim 44, 47, 48 remain indefinite because PGCs isolated from an embryo later than stage 14 as claimed are not distinguished from PGCs isolated from a stage 10 or stage 14 embryo. PGCs isolated from stage 10, 14 and after stage 14 embryos have the same function as supported by Petitte (of record, Development, 1990, Vol. 108, pg 185-189) who found cells capable of contributing to the germline in whole stage X embryos (pg 185, col. 2, second and third full sentences) and Ponce de Leon (of record, cited above) who obtained PGCs from Stage 13-14 embryos (pg 99, col. 1, about halfway down), and Naito (of record, Mol. Reproduction and Develop., 1994, Vol.

37, pg 167-171), who obtained PGCs from Stage 13-15 embryos (pg 322, col. 1, "Preparation of PGCs for preservation"). It cannot be determined how PGCs isolated from stage 10 and 14 and after stage 14 as claimed differ structurally or differ in the types of undifferentiated cells they produce. Applicants have not pointed to one specific structural or functional difference between PGCs isolated from stage 10 and 14 and after stage 14. As such, the metes and bounds of PGCs having the same structure and function as PGCs isolated after stage 14 cannot be determined.

D. Claims 44, 47, 48, 52-54 and 58 stand rejected under 35 U.S.C. 102(b) as being anticipated by Chang (1995, Cell Biol. Internatl. Vol. 19. No. 2, pg 143-149) for reasons of record.

Chang (1995) isolated the germinal ridge of day 5 embryos (stage 27-28) and cultured the cells for 4 or 5 days (pg 143, "Preparation of germinal ridge and culture of stroma cells"; pg 146, Fig. 2 and caption for Fig. 2). The germinal ridge cells comprised stromal cells (pg 144, line 6) and PGCs (last sentence on pg 144: "The feeder layer derived from GRs must contain intrinsic PGCs"). It is noted that applicants used the same method (pg 13, line 22). The germinal ridge cells were cultured in conditioned media comprising LIF, IGF and FGF-b (pg 144, col. 1, 1st full ¶). The germinal ridge cells in conditioned media were used as a "preconditioned feeder matrix" (pg 144, col. 1, third paragraph: "They were then used as feeder cells for the culture of PGCs collected from 2-day old embryonic blood"; pg 144, col. 1, fourth paragraph: "GR stroma cells suspended in 100 µl of culture medium were also seeded in wells of 96-well culture plates and incubated for at least 4 days before culture with 5-day-old PGCs"). The

germinal ridge cell PGCs in the stromal cells were distinguished from the 2-day blood PGCs: "The feeder layer derived from GRs must contain intrinsic PGCs. To distinguish 2-day PGCs from these 5-day PGCs probably contained in the GR stroma cell layer, we labeled..." (last three lines on pg 144 and first line on pg 145).

"Undifferentiated cells expressing an ES phenotype" encompasses undifferentiated cells having a large nucleus, a prominent nucleolus and little cytoplasm, including PGCs. PGCs have a large nucleus, a prominent nucleolus and little cytoplasm because the metes and bounds of what applicants consider "large," "prominent," and "little" are unclear and because the nucleus, nucleolus and cytoplasm of PGCs are larger, more prominent and smaller, respectively, than in other types of cells. The phrase also encompasses undifferentiated cells able to contribute to germ cells upon being introduced into a recipient embryo. PGCs express an ES phenotype because they are able to contribute to germ cells upon being introduced into a recipient embryo (see 112/2nd).

The 5-day culture of PGCs derived from PGCs found in the germinal ridge stromal cells (pg 145, Table 2) have "an embryonic stem cell phenotype" because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells and because they are able to contribute to germ cells upon being introduced into an embryo.

The 5-day culture of PGCs is "derived from the chicken [PGCs]" as claimed because they are formed or developed from the PGCs in the original germinal ridge cell

isolate. Table 2, on pg 145, explicitly compares the number of PGCs in the germinal ridge cell stromal feeder layer on days 1 and 5.

The patent office does not have the means to compare the size of the germinal ridge PGCs in culture after 5 days to the PGCs contained in the original germinal ridge isolate. Furthermore, PGCs size varies, and the size at which cells are "smaller than the chicken primordial germ cells" varies. Therefore, without evidence to the contrary, some of the 5-day germinal ridge PGCs are smaller than PGCs in the original germinal ridge isolate, thus meeting the phrase "smaller than the chicken primordial germ cells" as claimed.

The 5-day culture of PGCs aggregated (pg 146, Fig. 2d and caption for Fig. 2d), which is equivalent to "colonies of tightly packed undifferentiated chicken cells" as claimed.

Claims 53 and 54 are included because the structure and function of a culture in which the ES cell phenotype is maintained for 5 days is equivalent to a culture in which the ES cell phenotype is maintained for one or two months. The 5-day culture of PGCs derived from PGCs found in the genital ridge of a day 5 embryo described by Chang (1995) has the same structure and function as cells in which the ability of PGCs to contribute to the germline is maintained for one or two months. Culturing the PGCs for one or two months does not alter the structure or function of the culture. Thus, the limitations in claims 53 and 54 do not distinguish the structure or function of the cells within the culture claimed from those of Chang (1995).

The germinal ridge cells inherently comprise fibroblasts as in claim 58 because the cells were grown in fibroblast growth factor (pg 144, first full paragraph).

In the alternative, PGCs isolated from the heart or vitelline vein of stage 13-14 chicks were obtained and added to the germinal ridge stromal cell culture (pg. 144, "Preparation and culture of PGCs"). The PGCs isolated from the heart or vitelline vein of stage 13-14 chicks have the same structure and function as PGCs isolated from the germinal ridge or gonad of chicken embryos at a stage greater than 14 as claimed. The process limitation of isolating cells from a genital ridge or gonad after a stage greater than stage 14 in claim 44 does not bear patentable weight on the product claimed because it is a process step that does not distinguish the structure or function of the cells isolated over those described by Chang (1995). I.e. PGCs capable of deriving undifferentiated chicken cells having an ES phenotype as claimed can be isolated by means other than from a germinal ridge or gonad after stage 14 as claimed. In addition, the limitation of isolating PGCs and stromal cells together does not bear patentable weight because PGCs and stromal cells isolated separately then mixed together have the same structure and function as those isolated together.

"Undifferentiated cells expressing an ES phenotype" encompasses PGCs for the reasons above in this rejection (see also 112/2$^{nd}$) because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells and are able to contribute to germ cells upon being introduced into an embryo.

The 5-day embryonic blood PGCs are "derived from the chicken [PGCs]" as claimed because they are formed or developed from the PGCs found in the embryonic

blood isolate. Table 1, on pg 145, explicitly compares the number of PGCs isolated from embryonic blood in culture on days 1 and 5.

The patent office does not have the means to compare the size of the blood PGCs in culture after 5 days to the PGCs contained in the original blood isolate. Furthermore, PGCs size varies, and the size at which cells are "smaller than the chicken primordial germ cells" varies. Therefore, without evidence to the contrary, some of the 5-day blood PGCs are smaller than PGCs found in the isolated embryonic blood, thus meeting the phrase "smaller than the chicken primordial germ cells" as claimed.

The 5-day blood PGCs aggregated (pg 146, caption of Fig. 1e, pg 147, Fig. 1e), which is equivalent to "colonies of tightly packed undifferentiated chicken cells" as claimed.

E. Claims 44, 47, 48, 52-54 and 58 stand rejected under 35 U.S.C. 102(b) as being anticipated by Chang (1997, Cell Biol. Internatl., Vol. 21, No. 8, pg 495-499) for reasons of record.

Chang (1997) isolated germinal ridge stromal cells (GRSC) from day 5 (stage 27-28) embryos and cultured the cells for 5 days in media containing IGF, FGF and LIF. Day 5 embryos are stage 27 (pg 496, "Preparation and culture of gPGCs", line 2). Chang (1997) isolated gPGCs from the 5-day GRSC culture and injected them into recipient embryos. The gPGCs provided germline transmission (pg 496, "Preparation and culture of gPGCs"; pg 497, Fig. 1, "Progeny of germline chimeric chickens"). The GRSCs isolated by Chang (1997) inherently comprised PGCs as evidenced by Chang

(1995) [sic] who taught day 1 cultures of GRSC comprised PGCs (pg 145, Table 2). The adhesive cells of the 5-day GRSC culture (last sentence of "Preparation and culture of gPGCs") are a "preconditioned feeder matrix" because they were established in culture prior to the removal of gPGCs. The media in the day-5 culture was "conditioned" because it contained biologically active components obtained over time. The media had LIF, IGF and FGF-b (pg 496, "Preparation and culture of gPGCs").

"Undifferentiated cells expressing an ES phenotype" encompasses undifferentiated cells having a large nucleus, a prominent nucleolus and little cytoplasm, including PGCs. PGCs have a large nucleus, a prominent nucleolus and little cytoplasm because the metes and bounds of what applicants consider "large," "prominent," and "little" are unclear and because the nucleus, nucleolus and cytoplasm of PGCs are larger, more prominent and smaller, respectively, than in other types of cells. The phrase also encompasses undifferentiated cells able to contribute to germ cells upon being introduced into a recipient embryo. PGCs express an ES phenotype because they are able to contribute to germ cells upon being introduced into a recipient embryo (see 112/2nd). Accordingly, PGCs derived from the culture (pg 496, "Preparation and culture of gPGCs"; pg 497, Fig. 1, "Progeny of germline chimeric chickens") are "undifferentiated chicken cells expressing an embryonic stem cell phenotype" because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells and are able to contribute to germ cells upon being introduced into an embryo.

The PGCs used to make the germline chimeras are "derived from the chicken [PGCs]" as claimed because they are formed or developed from PGCs found in the original GRSC isolate.

The patent office does not have the means to compare the size of the resulting 5-day gPGCs to the PGCs in the original GRSC isolate. Furthermore, PGCs size varies, and the size at which cells are "smaller than the chicken primordial germ cells" varies. Therefore, without evidence to the contrary, some of the 5-day gPGCs are smaller than PGCs in the original GRSC isolate, thus meeting the phrase "smaller than the chicken primordial germ cells" as claimed.

The cells had to be suspended by gentle pipetting (pg 496, col. 1, last sentence of "Preparation and culture of gPGCs"), which implies the cells were inherently "colonies of tightly packed" as claimed. "Tightly packed" encompassed any degree of clumping, more specifically, clumping that requires suspension by gentle pipetting.

Claims 53 and 54 are included because cells isolated from the genital ridge of a day 5 embryo and cultured as described by Chang (1997) do not differ from cells isolated and maintained as claimed, wherein the ES cell phenotype is maintained for one or two months as claimed. The structure and function of a culture of cells in which the ES cell phenotype is maintained for 5 days is equivalent to a culture in which the ES cell phenotype is maintained for one or two months. Culturing the cells for one or two months does not alter the structure or function of the culture. Thus, the limitations in claims 53 and 54 do not distinguish the structure or function of the cells within the culture or the components of the culture from those known in the art.

The GRSC inherently comprise fibroblasts as in claim 58 because the cells were grown in fibroblast growth factor (pg 496, col. 1, "Preparation...").

F. Claims 44, 47, 48, 51-54 and 56-58 stand rejected under 35 U.S.C. 102(e) as being anticipated by Petitte (US Patent 5,340,740), Petitte (US Patent 5,656,479) or Petitte (US Patent 5,830,510) for reasons of record.

In Example 4, Petitte taught, "several methods of culturing cell [sic] with an embryonic stem cell phenotype from unincubated chicken embryos were carried out [sic]. First..." (col. 7, lines 4-7, of '470) whole stage X chicken embryos were isolated, dissociated and seeded the cells onto a chicken embryonic fibroblast feeder layer in the presence of BRL conditioned medium. "Only a few clusters of cells remained relatively undifferentiated and contained large amounts of lipid" (col. 7, lines 7-14, of '740).

The cells isolated from stage X embryo taught by Petitte inherently had PGCs because cells isolated from stage X embryos are capable of becoming germline cells (Petitte, Development, 1990, Vol. 108, pg 185-189, of record; pg 185, col. 2, second and third full sentences). PGCs isolated from the genital ridge or gonad of an embryo at a stage greater than 14 as claimed are not structurally or functionally distinguished over the PGCs inherently isolated from whole stage X embryos described by Petitte.

"Undifferentiated cells expressing an ES phenotype" encompasses undifferentiated cells having a large nucleus, a prominent nucleolus and little cytoplasm. PGCs have a large nucleus, a prominent nucleolus and little cytoplasm because the metes and bounds of what applicants consider "large," "prominent," and "little" are unclear and because the nucleus, nucleolus and cytoplasm of PGCs are larger, more

prominent and smaller, respectively, than in other types of cells (see 112/2nd). Accordingly, the "clusters of cells" that "remained undifferentiated" in the first method of Example 4 "express an ES cell phenotype" as claimed because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells.

The "clusters of cells" in the first method of Example 4 also "express an ES cell phenotype" because Petitte implies the cells are ES cells. Petitte calls the cells in the first method of Example 4 ES cells in the title ("Culturing of Arian [sic] Embryonic Stem Cells"). Petitte calls the first method of Example 4 one of "several methods of culturing cells with an embryonic cell phenotype" (col. 7, lines 4-7, of '740). Petitte teaches the embryonic stem cell phenotype is a large nucleus, a prominent nucleolus and little cytoplasm (col. 2, lines 20-23, of '740). Therefore, the "clusters of cells" in the first method of Example 4 inherently have a small nucleus, a prominent nucleolus and little cytoplasm because Petitte implies they are ES cells.

The clusters of undifferentiated cells obtained were inherently "smaller" than PGCs and "tightly packed" as claimed because Petitte implied they were ES cells.

The undifferentiated cells described by Petitte are "derived from the chicken [PGCs]" as claimed because they are formed or developed from the PGCs inherently found in the original dissociated whole stage X embryo.

The process limitation of isolating PGCs and stromal cells together "from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14" as in claim 44 is not structurally distinct from the PGCs and stromal fibroblasts isolated separately described by Petitte.

Petitte mixed the stage X embryo cells with chicken fibroblast feeder cells (col. 7, lines 8-9, of '740) isolated from 10-day old chick embryos (col. 6, lines 21-24, of '740), which is "later than stage 14." The fibroblast feeder cells are "stromal cells" as claimed because they provide structural support (as opposed to parenchymal cells). The fibroblast feeder cells taught by Petitte are preconditioned because they are grown in media before the stage X embryo cells are added. Stromal cells isolated from the "gonad" or "genital ridge of an embryo" later than stage 14 as in claims 47 and 48 are not structurally or functionally distinguished over the fibroblast feeder cells harvested from 10-day old chicken embryos as taught by Petitte.

A culture in which the ES cell phenotype is maintained for one or two months as claimed (claims 53, 54) does not differ from a culture in which the ES cell phenotype is maintained for a number of passages as taught by Petitte because their structure and functions are equivalent. The process of maintaining the ES cell phenotype for one or two months does not distinguish the structure or function of the undifferentiated cells as compared to the culture comprising undifferentiated cells taught by Petitte.

It is not readily apparent whether the whole stage X embryo described by Petitte comprised fibroblasts or other stromal cells. The patent office does not have the ability to determine whether the whole stage X embryos described by Petitte have fibroblasts or other stromal cells or whether such fibroblasts or stromal cells would be structurally or functionally equivalent to fibroblasts/stromal cells isolated from the gonad or genital ridge of an embryo after stage 14 as claimed. Accordingly, the second method in Example 4 (col. 6, line 66, through col. 7, line 18, of '740) and the method of Example 5

(col. 7, lines 34-48, of '740) have not been included because it is not readily apparent that cells isolated from whole stage X embryos have chicken stromal cells as claimed, specifically stromal cells having the same structure as those isolated from the germinal ridge or gonad of an embryo after stage 14 as claimed.

The first method of Example 4 is in col. 6, lines 43-54, of '479 and col. 6, line 54-65, of '510.

G. Claims 44, 47, 48, 52-54 and 58 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Ponce de Leon (US Patent 6,156,569) in view of Chang (1995, Cell Biol. Internat'l, Vol. 19, page 143-149) for reasons of record.

Claims 51, 56 and 57 have been withdrawn from the rejection in view of applicants' arguments.

Ponce de Leon isolated PGCs from the dorsal aorta of stage XIV chicken embryos. The cells were cultured with complete medium, LIF, FGF, IGF and SCF for at least 25 days (col. 7, line 43 through col. 8, line 53).

"Undifferentiated cells expressing an ES phenotype" encompasses undifferentiated cells having a large nucleus, a prominent nucleolus and little cytoplasm, including PGCs. PGCs have a large nucleus, a prominent nucleolus and little cytoplasm because the metes and bounds of what applicants consider "large," "prominent," and "little" are unclear and because the nucleus, nucleolus and cytoplasm of PGCs are larger, more prominent and smaller, respectively, than in other types of cells. The phrase also encompasses undifferentiated cells able to contribute to germ cells upon being introduced into a recipient embryo. PGCs express an ES phenotype

because they are able to contribute to germ cells upon being introduced into a recipient embryo (see 112/2nd).

The PGCs described by Ponce de Leon are "undifferentiated chicken cells expressing an embryonic stem cell phenotype" because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells and because they are able to contribute to germ cells and somatic cells upon being introduced into an embryo (pg 100, col. 1, "Progeny tests" "Results and Discussion"; germline chimeras with speckled feathers are also somatic chimeras).

The undifferentiated cells that contributed to somatic and germ cells upon being introduced into an embryo were derived from the original PGCs in culture, which is equivalent to undifferentiated cells "derived from the chicken [PGCs]" as claimed because they formed or developed from PGCs in the original embryonic blood isolate.

The patent office does not have the means to compare the size of the PGCs used to make germline chimeras. Furthermore, PGCs size varies, and the size at which cells are "smaller than the chicken primordial germ cells" varies. Therefore, without evidence to the contrary, some of the PGCs used to make germline chimeras are smaller than the PGCs in the embryonic blood, thus meeting the phrase "smaller than the chicken primordial germ cells" as claimed.

"[C]olonies of tightly packed" cells encompasses any number of cells having any degree of clumping. The cells described by Ponce de Leon are clumped (col. 8, lines 41-50), which is equivalent to "colonies of tightly packed" cells as claimed.

The limitation of collecting PGCs from a the genital ridge or gonad of a chicken embryo at a stage later than stage 14 does not distinguish the structure or function of the PGCs from those isolated from the blood of stage 14 embryos as described by Ponce de Leon. Therefore, the PGCs isolated by Ponce de Leon are equivalent to PGCs isolated from the gonad or germinal ridge of a chicken embryo after stage 14 as in claim 44 because they produced undifferentiated cells that clump and are capable of creating a germline chimeric chicken. The limitation of collecting PGCs from a chicken embryo later than stage 14 together with chicken stromal cells as claimed does not bear patentable weight on the product claimed because it is a process step that does not distinguish the structure or function of the combination of PGCs and stromal cells.

Ponce de Leon did not teach the cultured also comprised chicken stromal cells isolated from the gonad or germinal ridge of a chicken embryo after Stage 14 or a preconditioned fibroblast feeder matrix.

However, Chang (1995) cultured PGCs with chicken stromal cells isolated from the germinal ridge of a chicken embryo of 5 day embryos (a stage later than stage 14) (pg 143, col. 2, "Preparation of germinal ridge and culture of stroma cells"; pg 144, col. 1, third full paragraph), which is equivalent to a preconditioned feeder matrix or to stromal cells isolated from the genital ridge of a chicken embryo at a stage later than stage 14. The limitation of isolating cells of the preconditioned feeder matrix from the gonad of a chicken embryo after stage 14 (claim 47) does not distinguish the feeder cells claimed over the cells isolated from the germinal ridge described by Chang (1995), i.e. feeder stromal cells obtained from cells isolated from the genital ridge of a 5 day old

embryo taught by Chang (1995) have the same structure as those isolated from the gonad of an embryo at a stage later than stage 14 as claimed. The stromal cell feeder layer described by Chang (1995) is a preconditioned feeder matrix (claim 44) because the cells were conditioned in various growth factors before being used as feeder cells (pg 144, col. 1, second and third full paragraphs). The stromal cell feeder layer described by Chang (1995) inherently comprises a fibroblast feeder matrix (claim 58) because the cells were preconditioned in fibroblast growth factor (pg 144, col. 1, first full paragraph).

Thus, it would have been obvious to one of ordinary skill in the art at the time the invention was made to culture PGCs isolated from the dorsal aorta as described by Ponce de Leon with stromal cells isolated from the germinal ridge of an chicken embryo at a stage later than stage 14 as described by Chang (1995). One of ordinary skill in the art at the time the invention was made would have been motivated to culture the PGCs isolated from the dorsal aorta described by Ponce de Leon with chicken stromal cells isolated from the germinal ridge of an chicken embryo at a stage later than stage 14 as described by Chang (1995) because Chang (1995) taught the stromal cells increased the number of cells in culture (second and third sentences of abstract).

A culture in which the ES cell phenotype is maintained for one or two months as claimed (claims 53, 54) does not differ from a culture in which the ES cell phenotype is maintained "long-term" as taught by Ponce de Leon because their structure and functions are equivalent. The process of maintaining the ES cell phenotype for one or

two months does not distinguish the structure or function of the undifferentiated cells claimed over the PGCs taught by Ponce de Leon.

H. Claims 44, 47, 48, 51-54 and 56-58 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 8-10 of U.S. Patent No. 5,340,740 in view of the disclosure of '740 and Chang (1995, Cell Biol. Internat'l, Vol. 19, page 143-149) for reasons of record.

Claims 1 and 8-9 of '740 are directed toward a method of making a sustained culture of undifferentiated chicken cells having an ES cell phenotype maintained on a mouse fibroblast feeder layer. The methods claimed in '740 taken with the disclosure of '740 produce a culture equivalent in structure and function to the culture now claimed. In particular, claim 10 is drawn to a sustained culture that has the same structure and function as the culture now claimed. While claim 1 of '740 requires isolating cells from a blastoderm prior to formation of the primitive streak, isolating cells from the genital ridge or gonad of an embryo at a stage later than stage 14 as now claimed does not distinguish the culture produced in the method of claim 1 of '740 from the culture now in claim 44. A method claim in the instant application where cells were isolated from an embryo after stage 14 having the structure and function claimed would be patentably distinct from the method of claim 1 in '740; however, the product now claimed is not patentably distinct from the product made by the method of claim 1 or the product of claim 10 of '740. The product now claimed is an obvious variant of the product made in the methods claimed in '740 in view of the disclosure of '740 and the teachings of Chang (1995).

In Example 4, Petitte taught, "several methods of culturing cell [sic] with an embryonic stem cell phenotype from unincubated chicken embryos were carried out [sic]. First..." (col. 7, lines 4-7, of '740) whole stage X chicken embryos were isolated, dissociated and seeded the cells onto a chicken embryonic fibroblast feeder layer in the presence of BRL conditioned medium. "Only a few clusters of cells remained relatively undifferentiated and contained large amounts of lipid" (col. 7, lines 7-14, of '740).

The cells isolated from stage X embryo taught by Petitte inherently had PGCs because cells isolated from stage X embryos are capable of becoming germline cells (Petitte, Development, 1990, Vol. 108, pg 185-189, of record; pg 185, col. 2, second and third full sentences). PGCs isolated from the genital ridge or gonad of an embryo at a stage greater than 14 as claimed are not structurally or functionally distinguished over the PGCs inherently isolated from whole stage X embryos described by Petitte.

The "clusters of cells" that "remained undifferentiated" in the first method of Example 4 "express an ES cell phenotype" as claimed because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells.

The "clusters of cells" in the first method of Example 4 also "express an ES cell phenotype" because Petitte implies the cells are ES cells. Petitte calls the cells in the first method of Example 4 ES cells in the title ("Culturing of Arian [sic] Embryonic Stem Cells"). Petitte calls the first method of Example 4 one of "several methods of culturing cells with an embryonic cell phenotype" (col. 7, lines 4-7, of '740). Petitte teaches the embryonic stem cell phenotype is a large nucleus, a prominent nucleolus and little cytoplasm (col. 2, lines 20-23, of '740). Therefore, the "clusters of cells" in the first

method of Example 4 inherently have a small nucleus, a prominent nucleolus and little cytoplasm because Petitte implies they are ES cells.

The clusters of undifferentiated cells obtained were inherently "smaller" than PGCs and "tightly packed" as claimed because Petitte implied they were ES cells.

The undifferentiated cells described by Petitte are "derived from the chicken [PGCs]" as claimed because they are formed or developed from PGCs found in the original dissociated whole stage X embryo.

The process limitation of isolating PGCs and stromal cells together "from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14" as in claim 44 is not structurally distinct from the PGCs and stromal fibroblasts isolated separately described by Petitte.

Petitte mixed the stage X embryo cells with chicken fibroblast feeder cells (col. 7, lines 8-9, of '740) isolated from 10-day old chick embryos (col. 6, lines 21-24, of '740), which is "later than stage 14." The fibroblast feeder cells are "stromal cells" as claimed because they provide structural support (as opposed to parenchymal cells). The fibroblast feeder cells taught by Petitte are preconditioned because they are grown in media before the stage X embryo cells are added. Stromal cells isolated from the "gonad" or "genital ridge of an embryo" later than stage 14 as in claims 47 and 48 are not structurally or functionally distinguished over the fibroblast feeder cells harvested from 10-day old chicken embryos as taught by Petitte.

A culture in which the ES cell phenotype is maintained for one or two months as claimed (claims 53, 54) does not differ from a culture in which the ES cell phenotype is

maintained for a number of passages as taught by Petitte because their structure and functions are equivalent. The process of maintaining the ES cell phenotype for one or two months does not distinguish the structure or function of the undifferentiated cells as compared to the culture comprising undifferentiated cells taught by Petitte.

It is not readily apparent, and the patent office does not have the ability to determine whether the dissociated whole stage X embryo described by Petitte comprised fibroblasts or other stromal cells or whether such fibroblasts or stromal cells would be structurally or functionally equivalent to fibroblasts/stromal cells isolated from the gonad or genital ridge of an embryo after stage 14 as claimed. Accordingly, the second method in Example 4 (col. 6, line 66, through col. 7, line 18, of '740) and the method of Example 5 (col. 7, lines 34-48, of '740) have not been included in this portion of the rejection because they were not grown on a chicken stromal feeder layer.

Overall, the product claimed in the instant application has the same structure as the product made in the methods claimed in '740 and the product claimed in '740 in view of the disclosure of '740. The culture now claimed has the same structure and function as the one disclosed in the patent and would be covered by the patents. See *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). See also MPEP § 804.

In the alternative, the first and second methods of Example 4 and the method of Example 5 inherently isolate PGCs from stage X embryos that have the same structure and function as PGCs isolated from an embryo at a stage later than stage 14 as claimed for reasons cited above. The first method of Example 4 results in undifferentiated cells having an ES cell phenotype, "smaller," "colonies," "tightly packed"

and "derived from the PGCs" for reasons cited above. The second method of Example 4 and the method of Example 5 clearly result in undifferentiated cells having an ES cell phenotype that are smaller and form colonies as claimed because they result in cells having a large nucleus, prominent nucleolus and little cytoplasm (col. 7, lines 22-24, of '740) and closely packed nests of cells (Example 5, col. 7, lines 60-63, of '740). Thus, Petitte taught ES cells "derived from" the PGCs in the dissociated whole stage X embryo. The second method of Example 4 and the method of Example 5 did not teach culturing the dissociated stage X embryo cells (comprising PGCs) with chicken stromal cells.

However, at the time of filing, Chang (1995) cultured PGCs with chicken stromal cells isolated from the germinal ridge of a chicken embryo at a stage later than stage 14 (pg 143, "Preparation of germinal ridge and culture of stroma cells").

Thus, it would have been obvious to one of ordinary skill in the art at the time the invention was made to culture PGCs as described by Petitte with chicken stromal cells isolated from the germinal ridge of an chicken embryo at a stage later than stage 14 as described by Chang (1995). One of ordinary skill in the art at the time the invention was made would have been motivated to use chicken stromal cells isolated from the germinal ridge of an chicken embryo at a stage later than stage 14 as described by Chang to increase the number of PGCs as taught by Chang (1995) (abstract).

I. Claims 44, 47, 48 and 51-57 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of U.S. Patent

No. 5,656,479 or 5,830,510 in view of the disclosure of '479 or '510 and Chang (1995, Cell Biol. Internat'l., Vol. 19, page 143-149) for reasons of record.

Claim 1 of '479 and '510 are directed toward a sustained culture consisting essentially of undifferentiated chicken cells expressing an embryonic cell phenotype. Claim 2 states the cells may be cultured on STO feeder cells in the presence of LIF. The cells were isolated from the area pellucida of Stage X embryos and cultured on mouse STO cells. The cultures claimed in '479 and '510 taken with the disclosure of '479 and '510 are equivalent in structure and function to the culture now claimed.

In Example 4, Petitte taught, "several methods of culturing cell [sic] with an embryonic stem cell phenotype from unincubated chicken embryos were carried out [sic]. First..." (col. 6, lines 43-47, of '479; col. 6, lines 53-65, of '510) whole stage X chicken embryos were isolated, dissociated and seeded the cells onto a chicken embryonic fibroblast feeder layer in the presence of BRL conditioned medium. "Only a few clusters of cells remained relatively undifferentiated and contained large amounts of lipid" (col. 6, lines 51-54, of '479; col. 6, lines 62-64, of '510).

The cells isolated from stage X embryo taught by Petitte inherently had PGCs because cells isolated from stage X embryos are capable of becoming germline cells (Petitte, Development, 1990, Vol. 108, pg 185-189, of record; pg 185, col. 2, second and third full sentences). PGCs isolated from the genital ridge or gonad of an embryo at a stage greater than 14 as claimed are not structurally or functionally distinguished over the PGCs inherently isolated from whole stage X embryos described by Petitte.

The "clusters of cells" that "remained undifferentiated" in the first method of Example 4 "express an ES cell phenotype" as claimed because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells.

The "clusters of cells" in the first method of Example 4 also "express an ES cell phenotype" because Petitte implies the cells are ES cells. Petitte calls the cells in the first method of Example 4 ES cells in the title ("Culturing of Arian [sic] Embryonic Stem Cells"). Petitte calls the first method of Example 4 one of "several methods of culturing cells with an embryonic cell phenotype" (col. 6, lines 44-47, of '479; col. 6, lines 54-58, of '510). Petitte teaches the embryonic stem cell phenotype is a large nucleus, a prominent nucleolus and little cytoplasm (col. 2, lines 28-31, of '479; col. 2, lines 33-36, of '510). Therefore, the "clusters of cells" in the first method of Example 4 inherently have a small nucleus, a prominent nucleolus and little cytoplasm because Petitte implies they are ES cells.

The clusters of undifferentiated cells obtained were inherently "smaller" than PGCs and "tightly packed" as claimed because Petitte taught they were ES cells.

The undifferentiated cells described by Petitte are "derived from the chicken [PGCs]" as claimed because they are formed or developed from PGCs found in the original, dissociated whole stage X embryo.

The process limitation of isolating PGCs and stromal cells together "from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14" as in claim 44 is not structurally distinct from the PGCs and stromal fibroblasts isolated separately described by Petitte.

Petitte mixed the stage X embryo cells with chicken fibroblast feeder cells (col. 6, lines 47-49, of '479; col. 6, lines 58-60, of '510) isolated from 10-day old chick embryos (col. 5, line 66, through col. 6, line 2, of '479; col. 6, lines 7-10, of '510), which is "later than stage 14." The fibroblast feeder cells are "stromal cells" as claimed because they provide structural support (as opposed to parenchymal cells). The fibroblast feeder cells taught by Petitte are preconditioned because they are grown in media before the stage X embryo cells are added. Stromal cells isolated from the "gonad" or "genital ridge of an embryo" later than stage 14 as in claims 47 and 48 are not structurally or functionally distinguished over the fibroblast feeder cells harvested from 10-day old chicken embryos as taught by Petitte.

A culture in which the ES cell phenotype is maintained for one or two months as claimed (claims 53, 54) does not differ from a culture in which the ES cell phenotype is maintained for a number of passages as taught by Petitte because their structure and functions are equivalent. The process of maintaining the ES cell phenotype for one or two months does not distinguish the structure or function of the undifferentiated cells as compared to the culture comprising undifferentiated cells taught by Petitte.

It is not readily apparent, and the patent office does not have the ability to determine whether the dissociated whole stage X embryo described by Petitte comprised fibroblasts or other stromal cells or whether such fibroblasts or stromal cells would be structurally or functionally equivalent to fibroblasts/stromal cells isolated from the gonad or genital ridge of an embryo after stage 14 as claimed. Accordingly, the second method in Example 4 (col. 6, line 55, through col. 7, line 7, of '479; col. 6, line

66, through col. 7, line 18, of '510) and the method of Example 5 (col. 7, lines 23-37, of '479; col. 7, lines 34-49, of '510) have not been included in this portion of the rejection because they were not grown on a chicken stromal feeder layer.

Overall, the product claimed in the instant application has the same structure as the product claimed in '479 or '510 in view of the disclosure of '479 or '510. The culture now claimed has the same structure and function as the one disclosed in the patent and would be covered by the patents. See *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). See also MPEP § 804.

In the alternative, the first and second methods of Example 4 and the method of Example 5 inherently isolate PGCs from stage X embryos that have the same structure and function as PGCs isolated from an embryo at a stage later than stage 14 as claimed for reasons cited above. The first method of Example 4 results in undifferentiated cells having an ES cell phenotype, "smaller," "colonies," "tightly packed" and "derived from the PGCs" for reasons cited above. The second method of Example 4 and the method of Example 5 clearly result in undifferentiated cells having an ES cell phenotype that are smaller and form colonies as claimed because they result in cells having a large nucleus, prominent nucleolus and little cytoplasm (col. 6, lines 62-64, of '479; col. 7, lines 6-8, of '510) and closely packed nests of cells (Example 5, col. 7, lines 31-34, of '479; col. 7, lines 43-46, of '510). Thus, Petitte taught ES cells "derived from" the PGCs in the dissociated whole stage X embryo. The second method of Example 4 and the method of Example 5 did not culture the dissociated stage X embryo cells (comprising PGCs) with chicken stromal cells.

However, at the time of filing, Chang (1995) cultured PGCs with chicken stromal cells isolated from the germinal ridge of a chicken embryo at a stage later than stage 14 (pg 143, "Preparation of germinal ridge and culture of stroma cells").

Thus, it would have been obvious to one of ordinary skill in the art at the time the invention was made to culture PGCs as described by Petitte with chicken stromal cells isolated from the germinal ridge of an chicken embryo at a stage later than stage 14 as described by Chang (1995). One of ordinary skill in the art at the time the invention was made would have been motivated to use chicken stromal cells isolated from the germinal ridge of an chicken embryo at a stage later than stage 14 as described by Chang to increase the number of PGCs as taught by Chang (1995) (abstract).

Overall, the product claimed in the instant application has the same structure as the product made in the product claimed in '479 or '510 in view of Chang (1995). The culture now claimed has the same structure and function as the one disclosed in the patent in view of Chang (1995) and would be covered by the patents. See *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). See also MPEP § 804.

**(10) Response to Argument**

A. Applicants argue the examiner has not provided a preponderance of evidence that claims 53 and 54 as written are new matter. Applicants' argument is not persuasive. The examiner has cited the specification and provided a reasonable interpretation according to the plain language used in the and concluded the embodiment now claimed was not readily apparent from the teachings on pg 13-14 or anywhere else in the specification. "Culturing undifferentiated avian cells having an ES

cell phenotype" and "culturing avian embryo cells for at least one or two months" does not imply the ES cell phenotype is maintained for one or two months as claimed.

Applicants point to pg 1 and 3, which discuss undifferentiated cells having and ES cell phenotype; however, the citations do not contemplate or imply the ES cell phenotype is maintained for one or two months as claimed.

Applicants argue: "Given that the point of establishing the sustained cultures is not to generate long term cultures of PGCs but to establish derivatives of PGCs in culture that are undifferentiated and express an ESC phenotype, Appellants respectfully submit that the only reasonable interpretation for the cited passage is that PGCs are cultured under conditions sufficient to facilitate the survival and development of undifferentiated avian cells expressing an ESC phenotype from said PGCs" (pg 10 of Appeal Brief). Applicants point to Example 3, which describes culturing cells for 5 days. Applicants' arguments are not persuasive because they does not address the basis of the rejection. The pg 13-14 teaches culturing avian embryo cells having an ES cell phenotype and culturing avian embryo cells for one or two months but does not reasonably imply that the ES cell phenotype of the cultured avian embryo cells is maintained for one or two months as now claimed. Example 3 does not discuss whether an ES cell phenotype is maintained.

Applicants argue that maintaining cells that loose their undifferentiated state would be futile. Applicants' argument is not persuasive. The claims are not limited to maintaining the undifferentiated state for one or two months.

B. Applicants argue Ponce de Leon does not establish the state of the art because Ponce de Leon is limited to PGCs. Applicants' argument is not persuasive. The claims are not limited to sustaining ES cells having the ability to contribute to both germ and somatic cells upon being introduced into a recipient embryo. The PGCs described by Ponce de Leon are "undifferentiated chicken cells expressing an embryonic stem cell phenotype" because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells and because they are able to contribute to germ cells and somatic cells upon being introduced into an embryo (pg 100, col. 1, "Progeny tests" "Results and Discussion"; germline chimeras with speckled feathers are also somatic chimeras).

Applicants argue Ponce de Leon did not teach the conditions of LIF, bFGF, IGF and SCF that did provide long term culture of PGCs. Therefore, applicants conclude it is not possible to determine what LIF, bFGF, IGF and SCF conditions were necessary to sustain the culture for one or two months. Applicants' argument is not persuasive. Applicants essentially admit that based on the teachings of Ponce de Leon, one of skill could not determine what LIF, bFGF, IGF and SCF conditions were necessary to sustain the culture for one or two months. Applicants have not pointed to any teachings in the specification that overcome the limitations described by Ponce de Leon that are essential to maintain ES cells for one or two months as claimed. Ponce de Leon provides evidence that it would have required undue experimentation to determine the conditions required to maintain an ES cell phenotype for at least one or two months.

Applicants argue PGCs do not provide somatic cell chimerism and point to item 11 in the Declaration by Dr. Petitte. Applicants' argument and point 11 of the Declaration are not persuasive. First, and most importantly, the claims do not require obtaining undifferentiated cells having the ES cell phenotype of being able to contribute to germline and somatic cells. Second, the PGCs described by Ponce de Leon contribute to somatic cells because germline chimeras had speckled feathers (pg 100, col. 1, "Progeny tests" "Results and Discussion"). Germline chimerism is not the only chimerism that can result from the transfer of PGCs. The PGCs of Ponce de Leon also relate to the claim because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells.

Applicants argue Ponce de Leon and Petitte (1990) are not relevant to the claimed invention because they are limited to PGCs. Applicants' argument is not persuasive. Ponce de Leon obtained germline chimeras with speckled feather color (an indication of somatic cell chimerism) and Petitte (1990) obtained somatic and germline chimeras; therefore, the references are relevant to culturing undifferentiated cells having the ability to become somatic and germ cells upon being introduced into a recipient embryo. If Ponce de Leon and Petitte (1990) are limited to PGCs capable of contributing only to the germline, they are still relevant because the claims do not require obtaining cells capable of germline and somatic chimerism and may encompass deriving PGCs from PGCs (see 112/2$^{nd}$).

Overall, applicants' arguments regarding somatic chimerism are not persuasive because Van de Levoir taught ES cells derived from blastodermal cells could be

cultured for several weeks, but they were incapable of contributing to somatic tissues after more than 3 weeks in culture (pg 39, lines 1-9) and because the claims are not limited to obtaining cells capable of somatic chimerism.

Applicants argue the teachings in the specification are adequate to determine the parameters required to maintain ES cells for one or two months. Applicants' argument is not persuasive. First, the claims are not limited to obtaining ES cells. Second, the teachings in the specification are inadequate to overcome the teachings of Van de Levoir who states ES cells derived from blastodermal cells could be cultured for several weeks, but they were incapable of contributing to somatic tissues after more than 3 weeks in culture (pg 39, lines 1-9).

C. i) Applicants argue those of skill would understand from the specification that "undifferentiated cells having an ES cell phenotype" are morphologically distinguishable from PGCs. Applicants' argument is not persuasive. The sentence "embryonic stem cell phenotype refers to undifferentiated avian cells having a large nucleus, prominent nucleolus and little cytoplasm" (pg 9, lines 4-5) does not adequately define the distinguishing morphology of cells having an ES cell phenotype.

Applicants argue the description in the specification on pg 9 "would be understood to those of skill in the art as being a morphological characterization of ES cells and ES-like cells derived from PGCs." Applicants' argument is not persuasive. Those of skill would not know when applicants consider a nucleus "large," a nucleolus "prominent" or a cytoplasm "little." Those of skill would not know that applicants intended both ES cells and ES-like cells to be included by the phrase. Those of skill

would not know what applicants consider "ES-like" cells. Those of skill would not know whether to use the art-recognized definition of ES cells or the plain meaning of the phrase "an ES cell phenotype" instead of the ambiguous definition on pg 9 or how to incorporate the art-recognized definition of ES cells or the plain meaning of the phrase into the ambiguous definition on pg 9.

Overall, those of ordinary skill in the art would not be able to determine the metes and bounds of the cells encompassed by the phrase.

ii) Applicants argue the claims do not relate to PGCs per se. Applicants' argument is not persuasive. The claims require isolating PGCs after stage 14. It cannot be determined how PGCs isolated from stage 10 and 14 and after stage 14 as claimed differ structurally or differ in the types of undifferentiated cells they produce. Applicants have not pointed to one specific structural or functional difference between PGCs isolated from stage 10 and 14 and after stage 14.

Naito (Mol. Reproduction and Develop., 1994, Vol. 37, pg 167-171) was inadvertently omitted from the PTO-892 but has been part of the record because it has been available to view using PAIR.

D. Applicants argue Chang (1995) is limited to a culture of PGCs and did not culture undifferentiated cells derived from PGCs as claimed. Applicants argue Chang did not teach the cells derived from PGCs are tightly packed or are smaller than the PGCs. Applicants' arguments are not persuasive. The resultant germinal ridge PGCs or blood PGCs have "an embryonic stem cell phenotype" because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells

and because they are able to contribute to germ cells upon being introduced into an embryo. The resultant germinal ridge PGCs or blood PGCs are "derived from" PGCs in the original germinal ridge stromal cell isolate or the original blood isolate (Tables 1 and 2 (pg 145)). Both types of PGCs clump (Fig. 1e, Fig. 2d), which is equivalent to "colonies of tightly packed undifferentiated cells." The resultant germinal ridge PGCs or blood PGCs are "smaller than" the PGCs as claimed because PGCs size varies; therefore, some of the 5-day PGCs are smaller than PGCs in the original isolates.

Applicants argue Chang (1995) does not meet the limitations of claims 53 and 54 because Chang (1995) did not maintain the undifferentiated state for one or two months. Applicants' argument is not persuasive. First, claims 53 and 54 require maintaining an ES cell phenotype for one or two months, not maintaining an undifferentiated state for one or two months. Second, the structure and function of a culture of cells in which the ES cell phenotype is maintained for 5 days has the same structure and function as a culture in which the ES cell phenotype is maintained for one or two months. The limitations do not structurally or functionally distinguish the culture claimed from PGCs able to contribute to the germ line after 5 days in culture described by Chang (1995).

Applicants argue Chang (1995) does not meet the limitations of claim 58. Applicants' argument is not persuasive. All the limitations in claim 58 have been addressed in the basis of the rejection. The feeder layer described by Chang (1995) inherently comprises fibroblasts as claimed because the cells isolated were grown in fibroblast growth factor (pg 144, first full paragraph).

E. Applicants argue Chang (1997) is limited to a culture of PGCs and did not culture undifferentiated cells derived from PGCs as claimed. Applicants argue Chang (1997) did not teach the cells derived from PGCs are colonies of tightly packed cells or are smaller than the PGCs. Applicants' arguments are not persuasive. The resultant gPGCs have "an embryonic stem cell phenotype" because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells and because they are able to contribute to germ cells upon being introduced into an embryo. The resultant gPGCs are "derived from" PGCs in the original germinal ridge stromal cell isolate. The gPGCs inherently form "colonies of tightly packed" cells because they have to be suspended by gentle pipetting. The resultant gPGCs are "smaller than" the PGCs as claimed because PGCs size varies; therefore, some of the 5-day gPGCs are smaller than PGCs in the original germinal ridge isolate.

Applicants argue, "one of skill in the art would recognize that 'gentle pipetting without the use of digestive enzymes' would not release colonies of undifferentiated cells, which require the use of digestive enzymes to disrupt the colonies into individual cells." Applicants' arguments are not persuasive. One of skill would reasonably interpret the phrase "colonies of tightly packed differentiated cells" broadly; the phrase encompasses any number of cells clumped together. Those of skill would have easily recognized that gentle pipetting was adequate to disrupt a group of clumped cells. The claims do not clearly distinguish the number of cells in a colony or how tightly the cells are packed over the gPGCs suspended by gentle pipetting described by Chang (1997).

Applicants argue Chang (1997) does not meet the limitations of claims 53 and 54 because Chang (1997) did not maintain the undifferentiated state for one or two months. Applicants' argument is not persuasive. First, claims 53 and 54 require maintaining an ES cell phenotype for one or two months, not maintaining an undifferentiated state for one or two months. Second, claims 53 and 54 were included because a culture in which the ES cell phenotype is maintained for one or two months is not structurally or functionally distinguished from the culture in which the ES cell phenotype is maintained for 5 days described by Chang (1997).

Applicants argue Chang (1997) did not meet the limitations of claim 58. Applicants' argument is not persuasive. The GRSCs described by Chang (1997) inherently comprises fibroblasts as claimed because the cells isolated were grown in fibroblast growth factor (pg 496, col. 1, "Preparation...").

F. Applicants argue Petitte does not disclose the use of PGCs isolated from the gonad or genital ridge of an avian embryo at a stage later than stage 14. Applicants' arguments are not persuasive. The process limitation of isolating PGCs and stromal cells together from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14 does not distinguish the cells from PGCs of whole stage X embryos mixed with stromal fibroblasts isolated from a 10-day old chick embryo. PGCs isolated from the germinal ridge or gonad of an embryo after stage 14 as claimed are not structurally and functionally distinct from the PGCs of whole stage X embryos taught by Petitte. Stromal fibroblasts isolated from the germinal ridge or gonad as claimed are not structurally and functionally distinct from stromal fibroblasts isolated from a 10-day

old chick embryo. PGCs and stromal cells isolated together as claimed are not structurally or functionally distinct from PGCs and stromal cells isolated separately as taught by Petitte.

Applicants argue claims 47 and 48 should not be included because Petitte did not isolate feeder cells from the gonad or genital ridge of an embryo at a stage later than stage 14 as claimed. Applicants' argument is not persuasive. Stromal fibroblast feeder cells isolated from a 10-day old embryo are "later than stage 14" as claimed. Stromal fibroblast feeder cells isolated from a gonad or gential ridge are not structurally distinct from those isolated from a 10-day old embryo taught by Petitte.

G. Applicants argue Chang (1995) is limited to a culture of PGCs and did not culture undifferentiated cells derived from PGCs as claimed. Applicants argue Chang (1995) did not teach the cells derived from PGCs are colonies of tightly packed cells or are smaller than the PGCs. Applicants' arguments are not persuasive. Chang (1995) has not been relied upon for the PGCs or undifferentiated cells derived therefrom.

Applicants argue Ponce de Leon is limited to culturing PGCs and that the claim requires derivatives of PGCs. Applicants argue Ponce de Leon does not teach the PGC derivatives are smaller than PGCs and form tightly packed colonies as claimed. Applicants' arguments are not persuasive. The PGCs described by Ponce de Leon are "undifferentiated chicken cells expressing an embryonic stem cell phenotype" because they have a small nucleus, a prominent nucleolus and little cytoplasm as compared to other types of cells and because they are able to contribute to germ cells and somatic cells upon being introduced into an embryo (pg 100, col. 1, "Progeny tests" "Results and

Discussion"; germline chimeras with speckled feathers are also somatic chimeras). The resultant PGCs were "derived from" the PGCs in the original embryonic blood isolate. Some of the PGCs used to make germline chimeras are inherently smaller than PGCs in the original embryonic blood isolate because PGC size varies naturally. The resultant PGCs clumped (col. 8, lines 41-50), which is equivalent to "colonies of tightly packed" cells as claimed.

Applicants argue the PGCs of Chang (1995) in Fig. 2 grew individually or as aggregates, which appellants submit are not "tightly clumped" or "smaller" as claimed. Applicants' argument is not persuasive. Chang (1995) has not been relied upon for the PGCs or undifferentiated cells derived therefrom.

Applicants argue claims 47 and 48 should not be included because the claims do not require the four essential growth factors described by Ponce de Leon and conclude Ponce de Leon teaches away from using chicken fibroblast feeder cells. Applicants' arguments are not persuasive. Ponce de Leon has not been relied upon for the feeder cells. Chang (1995) isolated feeder cells from 5-day-old embryos, which are greater than stage 14 cultured in fibroblast growth factor ("Preparation of germinal ridge and culture of stroma cells"). Furthermore, claims 47 and 48 encompass using the four growth factors described by Ponce de Leon. Claims 47 and 48 do not require fibroblasts. Thus, applicants' conclusion that Ponce de Leon teaches away from using chicken fibroblast feeder cells is unfounded. Stromal cells conditioned in various growth factors before being used as feeder cells (pg 144, col. 1, second and third full paragraphs) are "preconditioned feeder cells" as claimed (claim 44).

Applicants argue claims 53 and 54 should not be included because the combined teachings of Ponce de Leon and Chang (1995) did not maintain the ES cell phenotype for one or two months as claimed. Applicants' argument is not persuasive. A culture in which the ES cell phenotype is maintained for one or two months as claimed does not differ from a culture in which the ES cell phenotype is maintained "long-term" as taught by Ponce de Leon because their structure and functions are equivalent. The process of maintaining the ES cell phenotype for one or two months does not distinguish the structure or function of the undifferentiated cells claimed over the PGCs cultured long-term taught by Ponce de Leon.

Applicants argue claim 58 should not be included because the combined references do not teach the limitations of claim 58. Applicants' argument is not persuasive. The stromal cell feeder layer of Chang (1995) inherently comprised fibroblasts because the cells were preconditioned in fibroblast growth factor (pg 144, col: 1, first full paragraph).

H. Applicants argue a prima facie case of obviousness has not been established because the examiner has not shown that one of ordinary skill in the art would have been motivated to employ PGCs isolated from the genital ridge or gonad of a later than stage 14 chicken in the methods of the '740 Patent even in view of Chang (1995). Applicants' argument is not persuasive. The process of isolating PGCs from an embryo after stage 14 does not structurally or functionally distinguish the PGCs claimed from the PGCs inherently contained in the dissociated whole stage X embryo described by Petitte. PGCs in the dissociated whole stage X embryo described by Petitte give rise to

undifferentiated cells that meet the limitations now claimed. Motivation to isolate PGCs from a stage after 14 is not required to obtain PGCs having the same structure and function as those claimed.

Applicants argue the combined teachings of Petitte and Chang (1995) do not provide a reasonable expectation of deriving undifferentiated cells having an ES cell phenotype as claimed from PGCs isolated from the genital ridge or gonad of a later than stage 14 chicken. Applicants' argument is not persuasive. The process of isolating PGCs from an embryo after stage 14 does not structurally or functionally distinguish the PGCs claimed from the PGCs inherently contained in the dissociated whole stage X embryo described by Petitte. Therefore a reasonable expectation of successfully isolating PGCs having the structure and function claimed from a stage after 14 is not required. The undifferentiated cells claimed can also be derived from PGCs inherently found in a dissociated stage X embryo as described by Petitte.

PGCs isolated as claimed are not structurally or functionally distinguished from the PGCs inherently isolated from whole stage X embryos taught by Petitte (see paragraph above). Therefore, motivation to isolate PGCs from an embryo at a stage later than 14 is not required to obtain PGCs having the same structure as those claimed.

Applicants argue the phrase "isolated together from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14" bears patentable weight because those of skill would not be motivated to perform such a method step. Applicants' argument is not persuasive. PGCs and stromal cells isolated together as

claimed are not structurally or functionally distinct from PGCs and stromal cells isolated separately as taught by Petitte in the first method of Example 4, or alternatively by the combined teachings of Petitte and Chang (1995).

Applicants argue those of skill in the art would have recognized that PGCs isolated after stage 14 would be incapable of forming the claimed sustained culture. Applicants argue the art taught away from isolating PGCs from an embryo after stage 14 as claimed. Applicants' argument is not persuasive. PGCs isolated from an embryo after stage 14 are not structurally or functionally distinguished from the PGCs inherently isolated from whole stage X embryos taught by Petitte (see paragraph above). Therefore, motivation to isolate PGCs from an embryo at a stage later than 14 is not required to obtain PGCs having the same structure as those claimed.

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). In this case, no hindsight reasoning was required to come to the claimed culture because PGCs isolated from a stage X embryo taught by Petitte combined with the stromal cells described by Petitte or those of Chang (1995) have the same structure and function as the culture now claimed.

Applicants argue the undifferentiated cells described by Petitte are not "derived from PGCs" or have "an ES cell phenotype" as claimed. Applicants argue an "ES cell phenotype relates to a morphological description of the culture undifferentiated cells that are recognized as being characteristic of ES cells. There is no evidence in the record that any cell isolated from stage X is morphologically similar to these cells." Applicants' arguments are not persuasive. The undifferentiated cells described by Petitte are "derived from the chicken [PGCs]" as claimed because they are formed or developed from the PGCs inherently found in the whole stage X embryo. The undifferentiated cells have an ES cell phenotype because they gave rise to "clusters of cells" that "remained undifferentiated" (col. 7, lines 11-14, of '740), because Petitte calls the cells ES cells (see title of Example 4, "Culturing of Arian [sic] Embryonic Stem Cells") and because Petitte calls the method the "first" of "several methods of culturing cells with an embryonic cell phenotype" (col. 7, lines 4-7, of '740).

Applicants argue the examiner has not provided adequate evidence that stromal cells are isolated in Example 5. Applicants' argument is not persuasive. Applicants appear to acknowledge that stromal cells **are** co-isolated with PGCs in whole stage X embryos as taught by Petitte. "Appellants respectfully submit that it is at elast [sic] equally likely that one of ordinary skill in the art would have believed that the stromal cells co-isolated with PGCs form the genital ridge/gonad microenvironment would... ...[not] be appropriate as feeder cells" (pg 39, first paragraph, of Appeal Brief). Furthermore, claim 44 does not require using stromal cells as feeder cells. Petitte cultured PGCs on chicken embryonic fibroblast feeder layers (first method of Example

4, col. 7, lines 8-10) which are stromal cells isolated after stage 14 having the same structure as those claimed. Alternatively the second method of Example 4 and the method of Example 5 of Petitte combined with Chang (1995) taught culturing cells from a whole stage X embryo on stromal cells isolated from the germinal ridge of an embryo after stage 14 as claimed.

Applicants argue they could not have claimed the subject matter now in the '740 patent at the time because it was not known that cells having an ES cell phenotype as claimed could be obtained from the gonad or genital ridge of a chicken embryo at a stage later than stage 14. Applicants' argument is not persuasive. The cultures claimed in the method of '740 or the product in '740 are not structurally or functionally distinguished from PGCs isolated from an embryo after stage 14 that give rise to undifferentiated cells having an ES cell phenotype as now claimed. Despite being obtained by a different method, the product claimed in the instant application is structurally and functionally the same as those disclosed in '740 and is covered by the claims in '740.

Applicants argue '740 does not teach isolating PGCs as claimed. Applicants' argument is not persuasive. The cells isolated from the stage X embryo taught by Petitte inherently had PGCs because Petitte (Development, 1990, Vol. 108, pg 185-189) of record, taught that cells isolated from freshly laid eggs as stage X embryos (pg 186, col. 1, lines 2-3) comprised cells that contributed to the germline (pg 185, sentence bridging col. 1-2 of abstract).

Applicants argue an ES cell phenotype is limited to a cells having a "large nucleus, prominent nucleolus, and little cytoplasm." Applicants' argument is not persuasive. While the specification states: "embryonic stem cell phenotype refers to undifferentiated chicken cells having a large nucleus, prominent nucleolus and little cytoplasm" (pg 9, lines 4-5), the description so ambiguous that the metes and bounds of the phrase are broader than just ES cells – the precise metes and bounds cannot be determined (see 112/2nd rejection). The specification also states an "'undifferentiated chicken cell expressing an embryonic stem cell phenotype' encompasses cells derived from chicken primordial germ cells and is therefore used to describe the cells cultured in accordance with the process of the present invention" (pg 9, lines 19-22). Thus, undifferentiated cells having an ES cell phenotype as claimed are not limited to undifferentiated chicken cells having a large nucleus, prominent nucleolus and little cytoplasm. Furthermore, cells isolated from stage X embryos described by Petitte resulted in undifferentiated cells having a large nucleus, prominent nucleolus and little cytoplasm (col. 7, lines 22-24, of '740) and closely packed nests of cells (Example 5, col. 7, lines 60-63, '740), which are equivalent to undifferentiated cells having the "ES cell phenotype" "derived from the chicken primordial germ cells" as claimed. In fact, Petitte calls the cells ES cells (see title of Example 4, "Culturing of Arian [sic] Embryonic Stem Cells" and of Example 5) and because Petitte calls the method of Example 4 the "first" and "second" of "several methods of culturing cells with an embryonic cell phenotype" (col. 7, lines 4-7, of '740).

I. Applicants argue the art teaches away from isolating PGCs after stage 14 to make undifferentiated cells having an ES cell phenotype as claimed. Applicants argue those of skill would not be motivated to isolate cells from an embryo after stage 14 as claimed. Applicants' arguments are not persuasive. The process of isolating PGCs from an embryo after stage 14 does not structurally or functionally distinguish the PGCs claimed from the PGCs inherently contained in the dissociated whole stage X embryo described by Petitte. Motivation to isolate PGCs after stage 14 is not required to obtain PGCs having the same structure and function as those claimed.

Applicants argue Chang (1995) is not relevant to the claims because it is limited to culturing PGCs per se. Applicants' argument is not persuasive. Chang (1995) has not been relied upon for teaching culturing PGCs. Petitte obtained undifferentiated cells having an ES cell phenotype from PGCs inherently found in the dissociated whole stage X embryo as taught by Petitte, wherein the undifferentiated cells are "smaller than" any of the PGCs inherently found in the dissociated whole stage X embryo and form "colonies of tightly packed" cells (see reasoning above in the basis of the rejection). It is noted that Chang (1995) does relate to culturing undifferentiated cells having an ES cell phenotype on chicken stromal cells isolated after stage 14 (see 102 rejection over Chang (1995) for reasoning).

**(11) Related Proceeding(s) Appendix**

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Michael C. Wilson

MICHAEL WILSON
PRIMARY EXAMINER

Conferees:

Peter Paras

PETER PARAS, JR.
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Ram R. Shukla

RAM R. SHUKLA, PH.D.
SUPERVISORY PATENT EXAMINER